The Life-Cycle of Cladocera, with Remarks on the Physiology of Growth and Reproduction in Crustacea.

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1. *Experiments on* Daphnia pulex.

In a paper on the life-cycle of *Moina rectirostris*, published in 1913 (5), it was shown by the late Mr. G. H. Grosvenor and myself that it was possible to inhibit entirely the production of the sexual forms by isolating the parthenogenetic parents soon after birth, and keeping them at a constant high temperature of 25–30° C. It was proved that for a succession of eight generations the isolated parents at this temperature gave no males or ephippial females, while parents of the same generations kept crowded at a temperature of about 14° C. or 5° C. gave about 50 per cent. males. We were unable to determine how the effect of isolation and crowding of the parthenogenetic parents influenced the production of the sexual forms, but two alternative suggestions were made, either that in the crowded glasses the animals were unable to obtain sufficient nutriment and were partially starved, or else that some excretory matter accumulated in the crowded glasses which influenced the production of males and sexual females.

In order to confirm the above results and to throw some light on the processes involved, breeding experiments have been carried on for some time with another species of Cladocera, the common *Daphnia pulex*. Mr. Robert Gurney very kindly gave me some dried mud from a pond which was known to contain the resting eggs of these animals, and, on placing the mud in a bowl of water, after some weeks some young Daphnia hatched out. One of these was kept until it had produced young, and the offspring of these young ones were used to start the first experimental generation.

D. pulex does not flourish on the food used for Moina, viz., manure infusion, but I had previously found that they could be cultivated with great ease if some green Alga, such as Protococcus, is added to the water in which they are kept. In order to have a constant supply of the Alga, stock cultures were made in a nutrient medium of inorganic salts to which a small amount of organic material was added. The best medium for growing the Protococcus was found to be a certain dilution of the mixture recommended by Miquel for growing Diatoms, which Mr. H. G. Thornton and myself have used for cultivating Euglena (8). By adding a pipette-full of the green growth to each glass in which the experimental animals are kept, it is possible

to ensure that there is always an excess of nourishment, because the culture consists only of the Alga and not of a mixed assemblage of bacteria, some of which may be useless as nourishment.

The scheme of the experiments is as follows :—In each generation a certain number of the individuals are isolated soon after birth in separate glasses; some of these are placed in an incubator at 27° C., others are stood in a water tank with circulating water at 10–17° C. Other individuals are kept crowded together in the same glass in numbers of 10–15, and of these crowded glasses some are again placed in the incubator and others in the circulating water at 10–17° C. Thus in each generation we have individuals subjected to four different conditions :—(1) Isolated at 27° C.; (2) crowded at 27° C.; (3) isolated at 10–17° C.; (4) crowded at 10–17° C. All are supplied with excess of Protococcus.

In Table I is given the result of breeding under these various conditions for eleven successive generations. This Table does not give the numbers of ephippial females which appeared among the parents, but it may be stated that ephippial or sexual females only appeared among the parents kept crowded at 10–17° C., which also gave a high percentage of males.

It will be noted that in this Table, besides the numbers of male and female offspring produced in each generation, a column is devoted to the number of parents, whether isolated or crowded, which were used for breeding. This factor, viz., the number of parents used, is one that must not be lost sight of, since, in order to prove that the production of males and sexual females is not simply a question of chance, it is clearly necessary to use a sufficient number of parents in each generation and under each condition to ensure that the effects of chance are ruled out. In as many cases as possible four broods were taken from each female. It was not found that there was any tendency for later broods to produce more sexual forms than early broods.

By consulting Table I it will be seen that neither in the isolated nor crowded individuals at 27° C. did any sexual forms appear throughout the eleven generations. Adding the totals of the isolated and crowded at 27° C. together we have, 90 + 150 = 240 parents gave 1188 + 643 = 1831 parthenogenetic females and no males.

Eighty-six individuals isolated at 10–17° C. gave 1860 parthenogenetic females and 56 males, or about 3 per cent. males, while 420 individuals crowded at 10–17° C. gave 3564 parthenogenetic females and 256 males, or about 7 per cent. males.

These results are in agreement with our previous experiments with Moina, showing that the effect of isolation and high temperature is to suppress the production of the sexual forms (5).

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Table I.

Generation.	Number of parents.	Number of offspring.		Number of parents.	Number of offspring.	
		Female.	Male.		Female.	Male.
		Isolated at 27° C.			Crowded at 27° C.	
1	5	35	0	20	47	0
2	13	43	0	20	65	0
3	13	80	0	—	—	0
4	4	14	0	10	20	0
5	1	22	0	16	28	0
6	11	103	0	20	171	0
7	12	331	0	16	147	0
8	9	253	0	24	105	0
9	7	120	0	16	20	0
10	6	75	0	8	40	0
11	9	112	0	—	—	—
Totals	90	1188	0	150	643	0
Generation.	Isolated at 10-17° C.			Crowded at 10-17° C.		
1	5	129	0	20	91	7
2	8	204	0	33	1035	100
3	10	319	0	70	482	15
4	10	157	0	27	242	8
5	9	168	0	30	190	0
6	7	144	0	65	415	0
7	3	49	0	75	452	36
8	10	250	0	30	470	65
9	11	253	56	15	40	0
10	6	99	0	30	87	25
11	7	88	0	25	60	0
Totals	86	1860	56	420	3564	256

Since it was found that the individuals crowded at 27° C. produced few young and did not flourish, the experiment was subsequently continued in a rather different way, only two kinds of conditions being employed, viz., isolation at 27° C. and crowding at 10–17° C. In this second experiment as nearly as possible equal numbers of parents in each generation were used in the isolated and crowded condition. Also a careful observation was kept to see how many of the parents used became ephippial or sexual. This experiment was made some time after the first with individuals that had been propagating by parthenogenesis, so that the first lot of parents used were about the 35th generation from the beginning of the cycle, i.e., the original winter egg from which the first individual emerged.

The result which is given in Table II shows that during eight successive generations 117 isolated parents at 27° C. produced 2564 parthenogenetic females, no males, and in no case became ephippial, while 129 crowded parents became ephippial in 17 cases and produced 1147 parthenogenetic females and 26 males.

Since nearly equal numbers of parents were used and more offspring were produced from the isolated parents than from the crowded, it is impossible to ascribe the production of sexual forms by the crowded individuals, and their entire absence in the case of the isolated parents, to chance.

If we add together the results for the isolated at 27° C. and for the crowded at 10–17° C. in the two experiments given in Tables I and II, we see that in nineteen generations 207 isolated parents at 27° C. gave 3752 parthenogenetic females and no males, while 549 crowded parents at 10–17° C. gave 4711 parthenogenetic females and 282 males, or about 6 per cent. males. It is also to be observed that, while no ephippial females appeared among the isolated parents at 27° C., about 10 per cent. of the crowded parents at 10–17° C. became ephippial.

Table II.

Genera-tion.	Isolated at 27° C.				Crowded at 10–17° C.			
	Number of parents.	Parents becoming ephippial.	Number of offspring.		Number of parents.	Parents becoming ephippial.	Number of offspring.	
			Female.	Male.			Female.	Male.
35	8	0	132	0	10	2	63	10
36	16	0	334	0	20	1	117	0
37	19	0	414	0	20	0	222	0
38	16	0	350	0	10	1	111	0
39	13	0	397	0	20	4	116	0
40	9	0	153	0	10	0	264	0
41	16	0	644	0	10	2	59	1
42	20	0	140	0	29	7	195	15
Totals......	117	0	2564	0	129	17	1147	26

The general result of the above records is to show that in *D. pulex*, as in *M. rectirostris*, it is possible to inhibit entirely the appearance of males and sexual females by isolating the parents soon after birth and keeping them at a temperature of 27° C. But if we look into the numbers given for each generation in Tables I and II, we find that the converse of the above statement does not hold good, *i.e.*, it is not the case that crowding at 10–17° C. always results in the production of the sexual forms. Thus, to

take some instances in Generation 6, 65 crowded parents gave 415 partheno-
genetic young and no males ; in Generation 37, 20 crowded parents gave 222
parthenogenetic young and no sexual forms. It must be concluded from this
that there is some factor involved in the production of the sexual forms other
than external conditions, viz., an internal factor. That this internal factor
is a regular rhythmical cycle, such as Weismann originally suggested (1), which
runs on without any regard to external conditions, is obviously not true, but
there is this very important element of truth in Weismann's view, namely,
that a species such as *D. pulex* never produces as many sexual forms per
cent. as a species like *M. rectirostris* ; and, as far as we know, no alteration of
the external conditions would make it do so. The facts suggest that for each
species of Cladoceran there is a maximal limit to the numbers in which sexual
forms may be produced, and that this number cannot be readily increased : but
it can be decreased, or entirely abolished, by external conditions such as isola-
tion and high temperature combined with abundant nutrition. If we suppose
with Woltereck (3) that the production of parthenogenetic and sexual forms
is due to the presence of two substances, a parthenogenetic substance and a
sexual substance, then we should say that the relative amounts of these
substances are fairly rigidly fixed for each species, and that, whereas the
amount of the sexual substance cannot be easily increased, its operation can
be indefinitely suspended by the action of external conditions. By this inter-
pretation of the facts it is possible to retain the really important part of
Weismann's theory, that the proportions in which the sexual forms are
produced in each species is fixed in its upper limit in accordance with the
adaptive necessities of the species, though we must maintain that these
proportions can be altered by the suppression of the sexual forms through
external conditions.

W. E. Agar (7) in a recent paper, after summarising the results of previous
workers, comes to the conclusion that there is no such thing as an internal
rhythm in Cladocera, and that the production of sexual or non-sexual forms
is entirely controlled by the environment. I am in agreement with Agar in
thinking that there is no hereditarily fixed rhythm, or that the production of
the sexual forms is rigidly fixed on to particular generations or particular
broods of these generations, but I find it impossible to believe that it is purely
due to environment that a species like *M. rectirostris*, under any conditions,
produces a far greater percentage of sexual forms than a species like *D. pulex*.
In other words, there is an internal factor concerned in the production of
the sexual forms ; this factor varies in different species of Cladocera ; its
operation can be entirely suspended by external conditions so that no sexual
forms are produced ; but there is no experimental justification for the view

that the production of sexual forms can be provoked at will in any or every generation of a particular species by alterations in the environment.

It is important to consider whether the factor of crowding can ever operate in a state of nature in the same way as under cultural conditions. There can be no doubt that the way this factor exerts its effect is through the presence of some excretory material in minute quantities, because in our cultures of Daphnia, which were fed on nothing but Protococcus, it was possible to ensure that there was in all cases an excess of food, so that the crowding could never cause a shortage of food. Since the animals were cultivated in glasses containing about 100 c.c. water, and the presence of 10 individuals constituted the normal crowded condition, it is clear that the reaction must be a very delicate one, due to the presence of extremely minute proportions of the substance in question. Now, in a state of nature, the small pools inhabited by many " polycyclic " species of Cladocera, *e.g.*, Moina, are often far more intensely crowded with individuals than under our cultural conditions. But, quite apart from small pools, it is frequently to be observed that large ponds are often so thickly populated with species like *D. magna* and *pulex* as to be coloured blood red, and I have met with cases where farmers have been afraid to water their horses at a pond on account of the extraordinary colour of the water. I have also observed that even in lakes, certain areas of water may be intensely crowded with some species of Cladocera, and it appears to me probable that the factor of crowding may play its part in the production of the sexual forms even in the largest bodies of water. The interesting report of Dr. Viktor Langhans on the Cladocera of the Hirschberg Lake in North Bohemia (4) shows that the various species of Cladocera inhabit for the most part quite localised areas of the lake, and, moreover, that the appearance of the sexual forms usually either coincides with or follows closely after the greatest activity in parthenogenetic reproduction, when crowding would be at its height.

2. *The Storage of Fat and Glycogen in its Relation to Growth and Reproduction in Cladocera.*

In the course of the breeding experiments described above, a contrast was noticeable between the individuals isolated at 27° C. and those kept crowded at a lower temperature. It was observed, even on inspection with the naked eye, that the young or fully grown individuals isolated at 27° C. were always of a pale, translucent green colour, while those crowded at the lower temperature were generally bright reddish orange, or, at any rate, showed a good deal of this colour. On examining the two kinds of individuals under the

microscope it was found that the reddish orange individuals owed their colour to the abundance of coloured fat globules present round the gut and ovary and at the bases of the limbs, while the pale green individuals were either entirely devoid of any fat or else possessed a few globules in the neighbourhood of the ovary. It was shown in a previous paper (6) that by placing living specimens of Cladocera, such as Moina or Daphnia, into a vessel of water in which a small quantity of neutral red is dissolved, it was possible to distinguish after a few hours certain bodies which took up the stain with great avidity. These bodies which stain intensely *intra vitam* with neutral red are distributed in three chief situations—(1) as very small granules in the polygonal cells of the chitogenous ectoderm (fig. 1) (in the case of ephippial females the chitogenous cells of the ephippium, which is formed of very thick chitin, contain much larger masses of glycogen, see fig. 2);

FIG. 1.   FIG. 2.

FIG. 1.—Chitinous areas on carapace, representing chitinogenous ectoderm cells, with small granules of glycogen stained with neutral red *intra vitam*.

FIG. 2.—Chitinous areas on carapace in region of formation of ephippium, showing large lumps of glycogen, stained with neutral red.
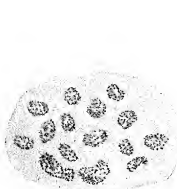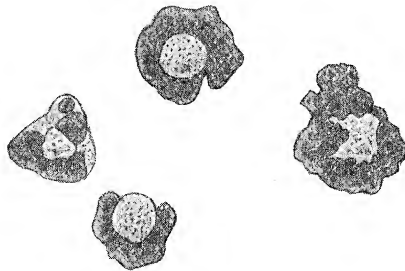

FIG. 3.

FIG. 4.

FIG. 3.—A group of cells from the gut, showing small glycogen granules occupying each cell, stained with neutral red.

FIG. 4.—Four large subcutaneous glycogen cells from base of limb, showing irregular masses of glycogen in periphery of cell, stained with neutral red.

(2) as small granules in the cells of the gut (fig. 3); and (3) as much larger, irregular-shaped masses in the connective tissue at the sides of the gut and at the bases of the limbs (figs. 4 and 5). There can be no doubt that these bodies are reserve material of the nature of glycogen, because the areas in which they occur are the same as those in which glycogen is known to occur in the higher Crustacea, and they exhibit the same appearance and staining reactions as the glycogen deposits in higher Crustacea. The fact that the so-called glycogen deposits of Crustacea stain so intensely *intra vitam*, and also after fixation with neutral red, suggests that they are not pure glycogen, or, at any rate, not identical with the glycogen found in the liver of warm-blooded animals, because neutral red does not show any particular affinity for these latter deposits. That they are largely composed of glycogen is shown, however, by their giving the iodine reaction both microchemically and, in the case of the higher Crustacea, after extraction with hot water in a test-tube reaction.
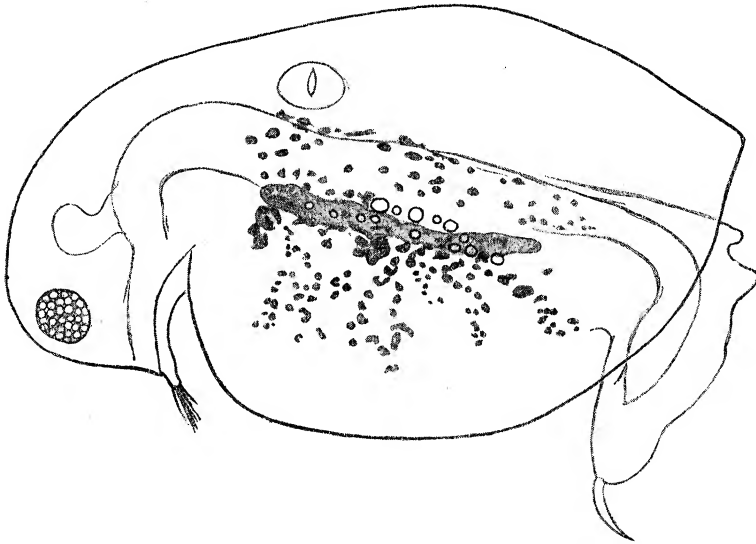

FIG. 5.—Parthenogenetic female, isolated at 27° C., showing reserve material present almost entirely as subcutaneous glycogen, with a few fat globules in neighbourhood of ovary.

It seems that the glycogen deposits in Crustacea consist of glycogen plus some nitrogenous material, probably a proteid derivative, which is responsible for the special affinity for neutral red. Leaving the exact chemical nature of these amylaceous deposits aside, it is to be observed that the pale green translucent individuals of *D. pulex* which have been kept isolated at 27° C. exhibit practically all their reserve substance in the form of this

glycogen material (fig. 5), while the reddish-orange individuals which have been crowded at a lower temperature have the greater part of this glycogen replaced by orange globules of fat. This does not apply to the small granules in the skin and gut, which are invariably present in all categories of individuals, but to the subdermal connective tissue masses round the gut and at the bases of the limbs. The connective tissue cells which store the reserve material have thus two alternatives: they may store glycogen, as in the case of the pale translucent individuals, or they may store preponderantly fat, as in the case of the crowded individuals at low temperatures.

By following the course of events occurring in the parthenogenetic females under the conditions of isolation at 27° C. and crowding at 10–17° C., it can be shown that the quite young individuals soon after birth in both cases have their reserve material distributed typically in the way shown in fig. 6. There
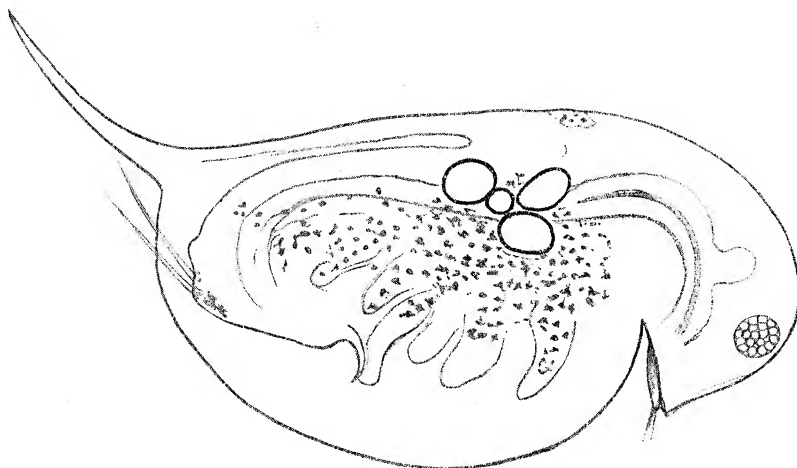

Fig. 6.—A young parthenogenetic female, showing distribution of glycogen and the few large fat globules.

are a few large globules of fat, represented by the dark circles, and a large supply of glycogen at the bases of the limbs. Now, as growth proceeds, the individuals kept isolated at 27° C. retain their reserve material in the form of glycogen and do not develop fat in any quantity (fig. 5); they grow and moult very rapidly and may reach maturity in three or four days. The individuals kept crowded at 10–17° C., on the other hand, tend to lose their glycogen deposits and to deposit large quantities of fat, and they grow and come to maturity much more slowly than the isolated individuals at 27° C. It is important to note here the coincidence of glycogen storage and rapid growth on the one hand, and of fat storage and retarded growth on the other.

Now the question arises, is it possible to connect this difference in behaviour

relative to reserve storage with the occurrence and non-occurrence of the sexual forms ?

An examination of the condition of the sexual forms strongly suggests that an affirmative answer can be given to this question. The ephippial females are always bright orange in colour owing to the abundant presence of fat in all the subdermal tissue at the sides of the gut and at the bases of the limbs, while the ephippial ovary is characterised by the presence of closely packed globules of fat in the eggs and nurse cells. The appearance of an ephippial female with its abundant reserve fat and opaque ovary loaded with fat is shown in fig. 7. In contrast with this the parthenogenetic female, even under the
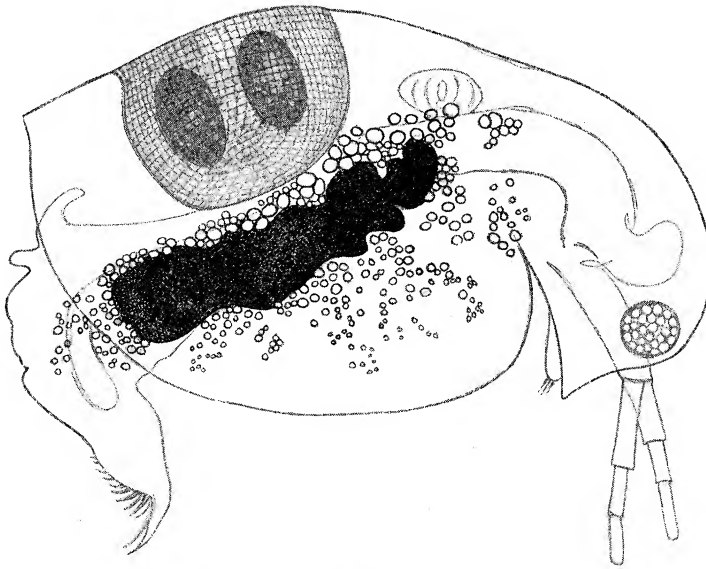

FIG. 7.—An ephippial or sexual female with ephippium of two egg chambers and opaque ovary full of fat. The reserve material present is in the form of very numerous fat globules of an orange-red colour.

crowded condition at low temperature, never exhibits so much fat in its reserve deposits, while the parthenogenetic ovary contains a very large quantity of amylaceous matter in addition to the comparatively sparse large fat globules in it. The adult males, as shown in fig. 8, resemble the ephippial females in the abundance of fat present as reserve substance.

Another point to be noted is that the ephippial females are inhibited in their growth and never attain to the same size as the parthenogenetic females kept isolated at 27° C., while the males are even more stunted.

We thus see that there is a remarkable coincidence between storage of glycogen and rapid growth on the one hand, and fat-storage and inhibition

of growth on the other ; that the parthenogenetic females which are kept crowded at low temperatures tend to store fat in place of glycogen and to be retarded in growth; that this tendency reaches its maximum in the sexual forms ; and that these sexual forms are produced only by the crowded parthenogenetic females which have a tendency to store fat and to be retarded in growth. The conclusion to be drawn from this series of facts is that the induced fat-storage and retarded growth of the parthenogenetic females crowded at low temperatures are the causal forerunners of the production of the sexual forms.

If we regard the parthenogenetic mode of reproduction as being essentially
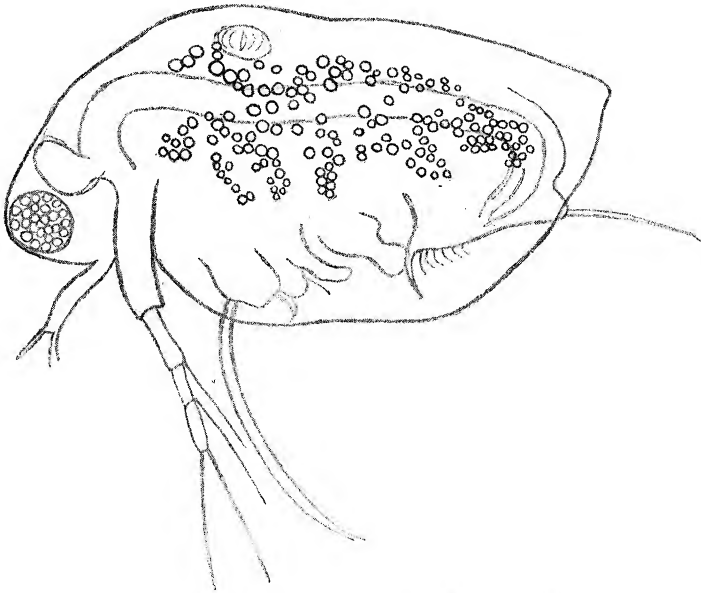
FIG. 8.—Adult, fortnight old, male, showing reserve material present in the form of fat.

a form of discontinuous growth or budding, we may observe that this is favoured by the conditions which also induce rapid growth in general, namely, preponderant storage of glycogen under the conditions of isolation and high temperature. The production of the sexual forms, which grow slowly and reproduce with extreme tardiness, is accompanied by a preponderant storage of fat, under the conditions of crowding and low temperature.

It is claimed, therefore, that the manner in which external conditions determine the continuance of parthenogenesis or the production of sexual forms is as follows : The condition of isolation and high temperature favours the storage of glycogen as opposed to fat, and this storage of glycogen leads

to rapid growth and to continuous parthenogenetic reproduction, which is to be looked upon as a mode of growth by budding. The condition of crowding and low temperature, on the other hand, stimulates the storage of fat as opposed to glycogen, and this storage of fat tends to inhibit growth and to call forth the production of the sexual forms of male and female, which are pre-eminently characterised by abundance of fat-storage and retarded growth and reproduction. Stated in a short and summary fashion, it is claimed that conditions which favour glycogen metabolism lead to rapid growth and parthenogenesis, while conditions which favour fat-metabolism lead to inhibition of growth and the production of sexual forms.

The way in which the factor of crowding leads to fat-storage, inhibition of growth, and the production of sexual forms is still somewhat obscure. But it is clear that the crowding does not act through partial starvation, because in all cases there was an excess of the food material present upon which the Daphnia were known to be feeding. This was ensured by feeding the animals on a pure culture of green Protococcus, which constituted the sole food of the organisms. The only other way in which crowding can be conceived to exert an effect is by the accumulation of some excretory product in the water as the result of the presence of numerous individuals. It is reasonable to suppose that this excretory matter might act in something the same way as phosphorus on a warm-blooded animal, namely, by stimulating the production of fat. All attempts at isolating or collecting this supposititious excretory matter have hitherto failed, and it would appear that it is easily destroyed, possibly by oxidation or bacterial action.

3. *The Storage of Fat and Glycogen in its Relation to Growth and Reproduction in Decapod Crustacea.*

We may now consider how far the theory of the connection between reserve-storage and growth and reproduction in the Cladocera harmonises with what we know of these processes in the higher Crustacea. Ever since the writings of Claude Bernard and the more recent work of Vitzou (2), it has been known that the growth and moulting of the higher Crustacea is accompanied by a remarkable heaping up of glycogen in the liver and subdermal connective tissue. If we take sections through the liver of a crab, such as *Carcinus mœnas*, which is about to cast its skin in the course of a day or two, it will be found, by staining the sections with iodine or neutral red, that the liver cells are crammed with small round granules of glycogen, to the exclusion of almost any other material (fig. 9). At this period there is practically no fat and the protoplasmic content of the cells is small. Besides these storage cells of the liver, the ferment cells, with darker protoplasm and larger nuclei, will be

seen. In addition to the greatly increased glycogen deposits in the liver, cells containing large masses of glycogen are abundant in the subdermal connective tissue and in the tissue between the liver cells.

If the liver of a crab in this condition is extracted for glycogen with hot potash solution, and the amount estimated as sugar by titration, the percentage of glycogen will be found to be very high, far higher than at any other time in the crab's life-history.

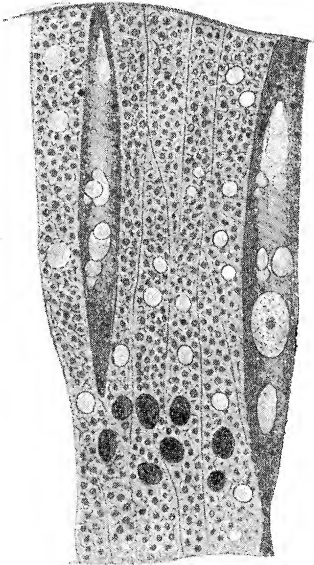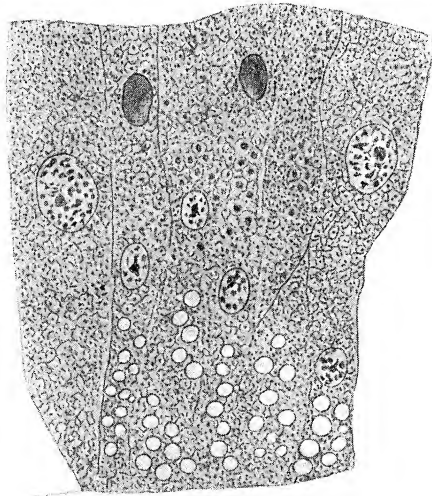If now we take sections through the liver of a crab that has recently

FIG. 9.   FIG. 10.

FIG. 9.—Section through portion of liver tube of Carcinus just about to moult. The storage cells are crammed with small round glycogen granules.

FIG. 10.—Section through portion of liver tube of ditto, some days after the moult. Reserve material is almost entirely absent from storage cells, which are full of protoplasm.

completed its moult and has the shell soft and flexible, we shall find it in the condition shown in fig. 10. The storage cells are now almost depleted of glycogen, and consist of protoplasm in which a few globules of fat, especially at the basal ends, are beginning to appear. The subdermal glycogen will also be found to have very much diminished in quantity. It is clear that the glycogen deposited in the liver and subdermal tissues just before the moult has been used up in the formation of the new skin and tissues during the rapid process of growth which follows the moult.

If, finally, we take sections through the liver of a hard-shelled crab at a period intermediate between two moults, when growth is not proceeding, we

obtain the appearance shown in fig. 11. Here the storage cells are seen to be filled with large and numerous fat globules, the only considerable stores of glycogen being found in the connective tissues outside the liver. Three such connective tissue cells with glycogen are shown in fig. 11. Quantitative estimations of the glycogen and fat in the liver and connective tissues under these various circumstances confirm the result obtained by histology, namely that during the moult there is abundance of glycogen (10 per cent.) and very little fat (3 per cent.), immediately after the moult there is very little glycogen
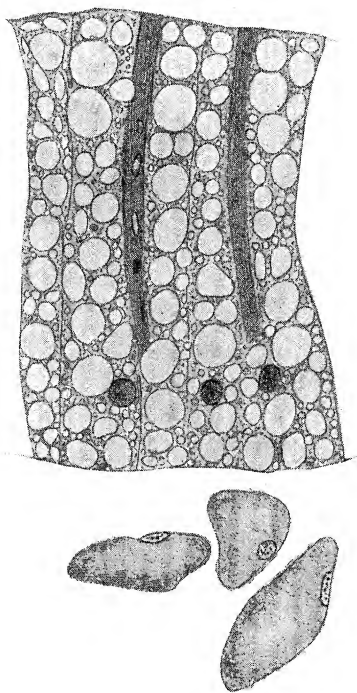

Fig. 11.—Section through ditto, about mid-way between two moults, when growth processes are in abeyance. The storage cells are crammed with fat globules. Three connective-tissue glycogen cells are shown outside the liver.

(0·1 per cent.) or fat (5 per cent.), and that between the moults there is abundance of fat (15 per cent.) and a rather small amount of glycogen (1·5 per cent. (The figures given here are only rough average approximations, but they give a trustworthy idea of the relative proportions of fat and glycogen in the liver under the various conditions.)

We conclude, therefore, that just as in the Cladocera, so in one of the higher Crustacea such as Carcinus, the period of active growth is accompanied by glycogen- as opposed to fat-metabolism, while the fat-storage in the liver

takes place in the intermediate periods when growth is in abeyance and the reproductive organs are maturing.

In *Carcinus mœnas* it was shown in a previous paper that the male and female differed in respect to their fat-metabolism (6). Thus in the female, maturing its ovaries, it was shown that the blood became flooded with lutein and fatty material to a much greater extent than the male, whose blood instead of becoming yellow with lutein is charged with the pink colouring matter tetronerythrin, but is not so heavily charged with fatty material as in the case of the female. Coincidently with this it was pointed out that the female does not grow to the same size as the male, and this is again probably due to the fact that the preponderant fat-metabolism of the female for the nourishment of the eggs exerts a check on growth. Finally it has been clearly shown that individuals of Carcinus infected with the parasite Sacculina do not increase in size at all after once the parasite has established its system of roots in the body of the crab, and this must certainly be ascribed to the fact that the Sacculina induces a most pronounced fatty habit in the liver of the crab, while the glycogenic function is permanently depressed. This has been completely proved by a series of quantitative estimations and histological examinations of the livers of infected crabs. The condition of the sacculinised crabs, both physiological and morphological, is converted by the action of the parasite into that of mature females in the act of ripening their ovaries, and this, as we have seen, consists in a pronounced fatty habit and the inhibition of growth.

The above considerations on the processes occurring in normal crabs and in those infected with Sacculina enable us to perceive that there is a marked agreement between these processes in the higher Crustacea and the conditions observed in the Cladocera. In both cases active growth (in which parthenogenetic reproduction is included) is accompanied by storage and use of glycogen for building up the new tissues and skin, while inhibition of growth and sexual reproduction is accompanied by storage and use of fat for the nourishment of the sexual products. This storage and use of fat is more pronounced in the case of the female than of the male, and it is clear that the fat-metabolism in the two sexes proceeds along different lines. In the case of the female the fatty material developed and stored in the metabolic organs is merely transferred across to the ovary, while in the case of the male it appears that the fat storage is less pronounced and that the fat is not transferred as such to the testis in anything like the same quantity, but is broken down and used for other purposes.

By thus bringing the processes of growth and reproduction in the Cladocera and in the higher Crustacea into agreement we obtain a certain

insight into the physiological basis of the antagonism between growth and sexual maturity which undoubtedly exists in the Crustacea, and the principle applies with modifications to organisms in general. This antagonism is seen to be due to the necessity for the mature organism to produce a special kind of nutriment for the reproductive organs, so that there is a corresponding lack of the suitable reserve substances for the purposes of growth. In the Crustacea and at some phase of the reproductive period in all organisms, the elaboration of fat for the supply of the ovary or accessory organs of reproduction is a marked feature of the metabolism in the mature female, and the diversion of reserve material in this form and for this purpose inhibits growth. In the male it is less obvious in what special form the reserve material for the nourishment of the reproductive organs is prepared, but here, again, it is probable that fat plays an important part, though the manner of its utilisation is certainly different from the comparatively passive transference which occurs in the female. The alteration in the metabolism thus brought about at sexual maturity, differing in its mode of operation in the male and female, we hold to be responsible for those morphological and physiological changes in the body which often accompany sexual maturity and are known as correlated secondary sexual characters.

The view developed here as to the nature of sexual maturity and its antagonism to growth has an interesting bearing on the meaning of sex in general. Speaking broadly, the onset of the sexual mode of reproduction in organisms occurs under conditions when continued growth or asexual multiplication is hindered either by lack of appropriate food or accumulation of excretory matter or by some internal weakening of the assimilative capacity. Under such conditions the organism responds by laying up reserve material for a special kind of resting reproductive cell instead of continuing to expend it in growth. The sexual mode of reproduction is thus a means of lying dormant during conditions unfavourable to continued growth. The differentiation into male and female may be looked upon as an economy or division of labour by which the female reproductive cell stores up compactly a mass of reserve material to be used for the nourishment of the next generation, but thereby loses the power of division, while the male reproductive cell retains the kinetic energy for division but relies on the female cell to supply the material for development.

Summary.

1. By isolating the young Daphnia at birth and keeping them at 27° C. it has been possible to breed them for 19 generations without the appearance of males or ephippial females, 3752 parthenogenetic females having been produced.

2. Parallel cultures to the above, when the parents are kept crowded to the number of 10 in a glass and at a temperature of 10–17° C., produced about 7 per cent. males and 10 per cent. ephippial females.

3. The crowding does not directly influence the supply of food, but appears to act by the accumulation of excretory matter in the glasses.

4. The parthenogenetic females kept isolated at 27° C. grow and reproduce more rapidly than those crowded at 10–17° C., and they store up reserve material almost exclusively in the form of glycogen, while the crowded parents at a lower temperature tend to store up fat instead of glycogen and are inhibited in their growth.

5. The storage of fat as opposed to glycogen is especially characteristic of the males and ephippial females ; hence it is judged that the fat-storage induced experimentally in the crowded parthenogenetic females at 10–17° C. is causally connected with the production by them of the sexual forms.

6. We may conclude that the habit of glycogen-storage leads to rapid growth and parthenogenesis, which is a form of discontinuous growth, while the habit of fat-storage leads to inhibition of growth and sexual mode of reproduction.

7. In the higher Crustacea the act of growth and moulting is accompanied by heaping up of glycogen in the liver storage-cells as opposed to fat, while in the periods between moults fat-storage preponderates.

8. Preponderant fat-storage in the liver is characteristic of female crabs maturing their ovaries and of crabs infected by Sacculina, and in both these cases growth is inhibited.

9. We thus find that both in Cladocera and Decapoda growth on the one hand, and sexual maturity on the other, are accompanied by a different type of reserve storage, which is also distinct in the case of the male and female. This is the physiological fact at the root of the antagonism between growth and sex.

10. Sexual reproduction is a reaction to conditions when continued growth is disadvantageous or impossible. Sexual differentiation is an economy or division of labour by which the female reproductive cell stores the material for development and thereby loses the power of division, while the male cell retains the power of division but relies on the female to supply the material for development.

LIST OF LITERATURE.

1. Weismann, A., " Beiträge zur Naturgeschichte der Daphnoiden," 'Zeitschrift f. Wiss. Zool.,' vols. 27-33 (1876-79).
2. Vitzon, A., " Recherches sur la Structure et la Formation des Tegumens chez les Crustacés décapodes," ' Arch. de Zool. Expér. et Génér,' vol. 10, p. 451 (1882).
3. Woltereck, R., " Veränderung der Sexualität bei Daphniden," ' Internationale Revue der Gesamten Hydrobiologie,' vol. 4 (1911).
4. Langhans, V. H., " Der Grossteich bei Hirschberg," ' Monographien zur Internationalen Revue der Gesamten Hydrobiologie,' vol. 3 (1911).
5. Grosvenor, G. H., and Smith, G., "The Life Cycle of *Moina rectirostris*," 'Quart. Journ. Micro. Sci.,' vol. 58, p. 511 (1913).
6. Smith, G., " Studies in the Experimental Analysis of Sex.—Part X," 'Quart. Journ. Micro. Sci.,' vol. 59, p. 267.
7. W. E. Agar, " Parthenogenetic and Sexual Reproduction in *Simocephalus vetulus* and other Cladocera," 'Journal of Genetics,' vol. 3 (1914).
8. Thornton, H. G., and Smith, G., " Conditions of Nutrition in Protozoa," ' Roy. Soc. Proc.,' B, June, 1914.

Lepidostrobus kentuckiensis, *nomen nov., formerly* Lepidostrobus Fischeri, *Scott and Jeffrey : a Correction.*

By D. H. Scott, For. Sec. R.S.

In a paper by Prof. Jeffrey and myself, published in the 'Philosophical Transactions,' last year,* we described a new species of *Lepidostrobus* from the Waverley Shale of Kentucky, under the name, *Lepidostrobus Fischeri.* My friend, Prof. R. Zeiller of Paris, has now kindly pointed out to me that the specific name *Fischeri* is not admissible, another fossil cone having been described in 1890 by M. B. Renault, under the same name, *Lepidostrobus Fischeri.*† I am sorry to have overlooked this reference, an oversight for which I am solely responsible.

Our fossil must now receive a new name and it is unfortunate that it is no longer possible to record in the specific designation the name of the discoverer, Mr. Moritz Fischer. The name I now propose for our cone is *Lepidostrobus kentuckiensis,* after the State in which the plant-bearing deposit occurs. The diagnosis is briefly repeated below.

* D. H. Scott and E. C. Jeffrey, "On Fossil Plants, showing Structure, from the Base of the Waverley Shale of Kentucky," 'Phil. Trans.,' B, vol. 205, pp. 315–373 (1914).

† "Études sur le Terrain Houiller de Commentry.—Flore Fossile, 2me partie," ' Bull. Soc. Industr. Min.,' 3e Série, IV, 2me Livr., p. 526, Plate 61, fig. 3 (1890).

Fig. 3.

Fig. 4.

Fig. 3.—A group of cells from the gut, showing small glycogen granules occupying each cell, stained with neutral red.

Fig. 4.—Four large subcutaneous glycogen cells from base of limb, showing irregular masses of glycogen in periphery of cell, stained with neutral red.

Fig. 5.—Parthenogenetic female, isolated at 27° C., showing reserve material present almost entirely as subcutaneous glycogen, with a few fat globules in neighbourhood of ovary.

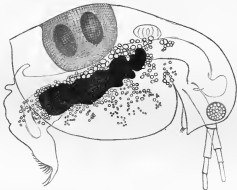Fig. 6.—A young parthenogenetic female, showing distribution of glycogen and the few large fat globules.

Fig. 7.—An ephippial or sexual female with ephippium of two egg chambers and opaque ovary full of fat. The reserve material present is in the form of very numerous fat globules of an orange-red colour.

Fig. 8.—Adult, fortnight old, male, showing reserve material present in the form of fat.

Fig. 9.—Section through portion of liver tube of *Carcinus* just about to moult. The storage cells are crammed with small round glycogen granules.

Fig. 10.—Section through portion of liver tube of ditto, some days after the moult. Reserve material is almost entirely absent from storage cells, which are full of protoplasm.

Fig. 11.—Section through ditto, about mid-way between two moults, when growth processes are in abeyance. The storage cells are crammed with fat globules. Three connective-tissue glycogen cells are shown outside the liver.